Journal of Clinical Periodontology
Official Journal of The European Federation of Periodontology
Founded by the British, Dutch, French, German, Scandinavian, and Swiss Societies of Periodontology

J Clin Periodontol 2008; 35: 1001–1010 doi: 10.1111/j.1600-051X.2008.01321.x

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# Alveolar ridge augmentation using implants coated with recombinant human bone morphogenetic protein-2: histologic observations

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*Wikesjö UME, Qahash M, Polimeni G, Susin C, Shanaman RH, Rohrer MD, Wozney JM, Hall J. Alveolar ridge augmentation using implants coated with recombinant human bone morphogenetic protein-2: histologic observations. J Clin Periodontol 2008; 35: 1001–1010. doi: 10.1111/j.1600-051X.2008.01321.x.*

***Abstract***

**Background:** Studies using ectopic rodent, orthotopic canine, and non-human primate models show that bone morphogenetic proteins (BMPs) coated onto titanium surfaces induce local bone formation. The objective of this study was to examine the ability of recombinant human BMP-2 (rhBMP-2) coated onto a titanium porous oxide implant surface to stimulate local bone formation including osseointegration and vertical augmentation of the alveolar ridge.

**Material and Methods:** Bilateral, critical-size, 5 mm, supra-alveolar, peri-implant defects were created in 12 young adult Hound Labrador mongrel dogs. Six animals received implants coated with rhBMP-2 at 0.75 or 1.5 mg/ml, and six animals received implants coated with rhBMP-2 at 3.0 mg/ml or uncoated control. Treatments were randomized between jaw quadrants. The mucoperiosteal flaps were advanced, adapted and sutured to submerge the implants for primary intention healing. The animals received fluorescent bone markers at weeks 3, 4, 7 and 8 post-surgery when they were euthanized for histologic evaluation.

**Results:** Jaw quadrants receiving implants coated with rhBMP-2 exhibited gradually regressing swelling that became hard to palpate disguising the contours of the implants. The histologic evaluation showed robust bone formation reaching or exceeding the implant platform. The newly formed bone exhibited characteristics of the adjoining resident Type II bone including cortex formation for sites receiving implants coated with rhBMP-2 at 0.75 or 1.5 mg/ml. Sites receiving implants coated with rhBMP-2 at 3.0 mg/ml exhibited more immature trabecular bone formation, seroma formation and peri-implant bone remodelling resulting in undesirable implant displacement. Control implants exhibited minimal, if any, bone formation. Thus, implants coated with rhBMP-2 at 0.75, 1.5 and 3.0 mg/ml exhibited significant bone formation (height and area) compared with the sham-surgery control averaging ($\pm$ SD) 4.4 $\pm$ 0.4, 4.2 $\pm$ 0.7 and 4.2 $\pm$ 1.2 *versus* 0.8 $\pm$ 0.3 mm; and 5.0 $\pm$ 2.2, 5.6 $\pm$ 2.2 and 7.4 $\pm$ 3.5 *versus* 0.7 $\pm$ 0.3 $mm^2$, respectively ($p < 0.01$). All the treatment groups exhibited clinically relevant osseointegration.

**Conclusions:** rhBMP-2 coated onto titanium porous oxide implant surfaces induced clinically relevant local bone formation including vertical augmentation of the alveolar ridge and osseointegration. Higher concentrations/doses were associated with untoward effects.

Key words: alveolar augmentation; bone morphogenetic protein; dental/oral implants; dogs; osseointegration; rhBMP-2; seroma; tissue engineering; titanium; titanium porous oxide

Accepted for publication 17 August 2008

**Conflict of interest and source of funding statement**

This study was supported by a grant from Nobel Biocare AB, Göteborg, Sweden. Jan Hall is an employee of Nobel Biocare AB. Rachel G. Sorensen was an employee of Wyeth Research at the time of study. John Wozney is an employee of Wyeth Research. Ulf M. E. Wikesjö was an employee of Wyeth Research, and serves as a consultant to Wyeth Research and Nobel Biocare AB.

A concept of applying bone morphogenetic proteins (BMPs) onto titanium surfaces for enhanced local bone formation has emerged from observations primarily in rodent ectopic and rodent and canine orthotopic models (Kawai et al. 1993, Herr et al. 1996, Cole et al. 1997, Esenwein et al. 2001, Vehof et al. 2001, Schmidmaier et al. 2002, Hartwig et al. 2003, Liu et al. 2005, 2007a, b, Becker et al. 2006, Hall et al. 2007). Theoretically, titanium implants coated with a bone inductive factor may stimulate local bone formation and osseointegration in regions with a need for supplementary bone augmentation procedures. In a step-wise progression, our laboratory has evaluated implant surface technologies, in particular titanium porous oxide implant surfaces, to serve as a vehicle for recombinant human BMP-2 (rhBMP-2). In an initial screening, titanium disks coated with rhBMP-2 were implanted into the ventral thoracic region in rats (Hall et al. 2007). The histologic evaluation showed significant bone formation and bone–implant contact (BIC) following a 14-day healing interval for all the surface technologies coated with rhBMP-2. A titanium porous oxide surface with open pores appeared as the most effective rhBMP-2 vehicle. Subsequently, screw-type implants with the titanium porous oxide surface with open pores coated with rhBMP-2 were implanted into the edentulated posterior mandible in dogs (Wikesjö et al. 2008b). Two concentrations of rhBMP-2, 0.2 and 4.0 mg/ml, were used. Peri-implant bone formation and implant osseointegration were evaluated following an 8-week healing interval. Implants coated with rhBMP-2 exhibited accelerated local bone formation in a dose-dependent order. A third study evaluated local bone formation and osseointegration at rhBMP-2-coated titanium porous oxide screw-type implants placed into Type IV bone in the posterior maxilla in the Cynomolgus monkey (Wikesjö et al. 2008a). The fluorescence microscopy evaluation showed robust bone formation at implants coated with rhBMP-2 at 2.0 mg/ml within 4 weeks, whereas bone formation at implants coated with rhBMP-2 at 0.2 mg/ml was modest. Collectively, these studies using ectopic and orthotopic, small and large animal models demonstrate that rhBMP-2 can be delivered successfully to induce local bone formation and osseointegration using a titanium porous oxide surface as a carrier. The objective of this study was to examine the potential of rhBMP-2 coated onto titanium porous oxide implant surfaces to stimulate local bone formation including osseointegration and vertical augmentation of the alveolar ridge.

## Material and Methods

### Animals

Twelve male Hound Labrador mongrel dogs, age 10–12 months, weight 20–25 kg, obtained from a USDA-approved dealer were used. Animal selection and management, surgery protocol and alveolar defect preparation followed routines approved by the local Institutional Animal Care and Use Committee. The animals were fed a canned soft dog-food diet throughout the study.

### Titanium implants

Implants with a titanium porous oxide surface (TiUnite™, ø 4.0 × 10 mm; Nobel Biocare AB, Göteborg, Sweden) were used. The implants, custom-made for the supra-alveolar peri-implant defect model, were manufactured with a reference notch 5 mm apical to the implant platform. The reference notch was designed to facilitate surgical placement leaving 5 mm of the implant in a supra-alveolar position and to serve as a reference point in the histologic and radiographic analysis. The sterile implants were coated with rhBMP-2 (Wyeth Research, Cambridge, MA, USA) at 0.75, 1.5 or 3.0 mg/ml, or left uncoated (control). Briefly, lyophilized rhBMP-2 and MFR 00169 buffer (5 mM glutamic acid, 5 mM sodium chloride, 2.5% glycine, 0.5% sucrose, 0.01% polysorbate 80, pH 4.5; Wyeth Research) was refrigerated at 4°C until use. Using aseptic technique, lyophilized rhBMP-2 was reconstituted with sterile water (Sterile Water for Injection, USP; Abbot Laboratories, North Chicago, IL, USA) to produce a 3.0 mg/ml solution. rhBMP-2 solutions at 0.75 and 1.5 mg/ml were prepared by diluting the rhBMP-2 solution reconstituted to 3.0 mg/ml liquid concentration with MFR 00169 buffer. Sterile implants were placed into sterile 0.5-ml wells (96 MicroWell™ Plates – Round Well Polypropylene, Nunc™ A/S, Roskilde, Denmark) and the wells were filled with 0.4 ml freshly prepared 0.75, 1.5 or 3.0 mg/ml rhBMP-2 solution to reach the implant platform. Implants were incubated in the rhBMP-2 solution for 30 min. and were then moved to air dry for a minimum of 6 h or overnight before implantation. All the preparations were performed in a Biogard, Class II, type A, laminar flow hood (Baker Company, Sanford, ME, USA) at room temperature.

### Surgery and experimental procedures

Food was withheld the night preceding surgery. The animals were pre-anesthetized with atropine (0.02–0.04 mg/kg), buprenorphine HCl (0.01–0.03 mg/kg), and acepromazine (0.2–0.3 mg/kg) IM. After tranquilization, an intravenous (IV) catheter was placed into the foreleg for induction with propofol (5–7 mg/kg IV). Animals were moved to the operating room and maintained on gas anesthesia (1–2% isoflurane/$O_2$ to effect). Conventional dental infiltration anesthesia was used at the surgical sites. The animals received a slow constant rate infusion of lactated Ringer's solution (10–20 ml/kg/h IV) to maintain hydration during surgery.

One experienced surgeon (U. M. E. W.) performed all the surgical procedures. Bilateral, critical-size, supra-alveolar, peri-implant defects were created in the mandibular premolar region (Figs 1–4; Wikesjö et al. 2006). Briefly, buccal and lingual mucoperiosteal flaps were reflected and alveolar bone removed around the circumference of the premolar teeth to a level approximately 6 mm apical to the cemento-enamel junction using water-cooled rotating burs. The premolar teeth were extracted and the first molar amputated at the level of the reduced alveolar crest. Three implants were placed into osteotomies prepared into the extraction sites of the third and fourth premolar teeth in each jaw quadrant. A few implants were

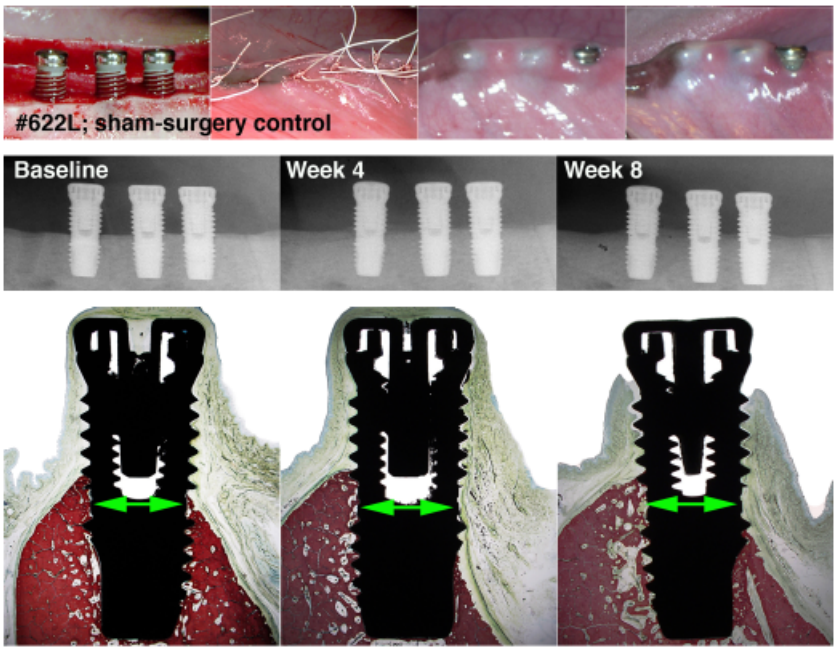


*Fig. 1*. Clinical photographs show sham-surgery control ø 4.0 × 10 mm implants following placement and wound closure, and healing at weeks 4 and 8. The implant platforms (cover screws) can be visualized through the mucosa at weeks 4 and 8 when one implant becomes exposed. Radiographs show limited new bone formation. The photomicrographs show limited bone formation confined to the lingual aspect of the implants, whereas the buccal aspect shows loss of crestal bone. Green arrows delineate the 5-mm notch placed level with the resident alveolar bone.

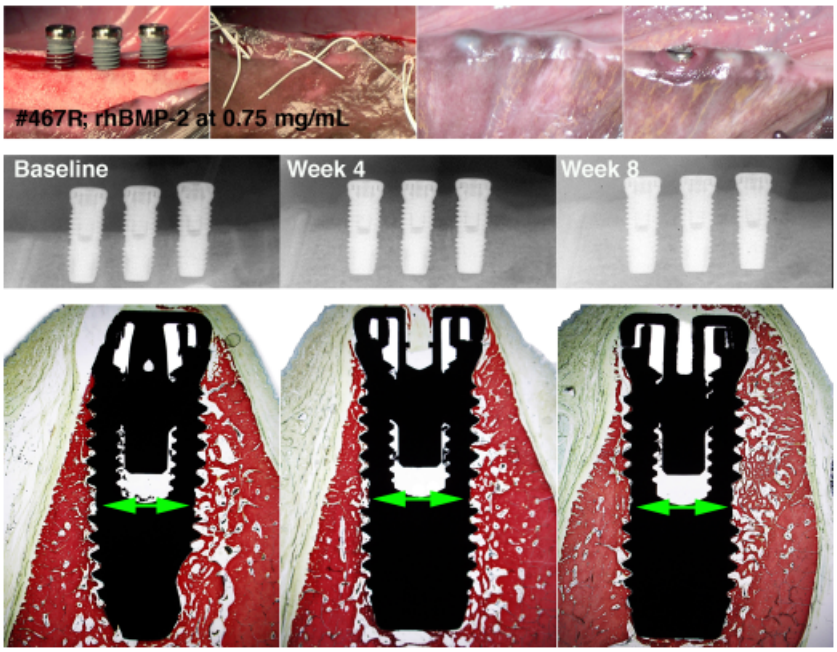


*Fig. 2*. Clinical photographs show ø 4.0 × 10 mm implants coated with recombinant human bone morphogenetic protein-2 (rhBMP-2) at 0.75 mg/ml following placement and wound closure, and healing at weeks 4 and 8. The implant platforms (cover screws) can be visualized through the mucosa at weeks 4 and 8 when one implant becomes exposed. Radiographs show bone formation reaching the implant platform at weeks 4 and 8. The photomicrographs show bone formation with an established cortex reaching or exceeding the implant platform. Green arrows delineate the 5-mm notch placed level with the resident alveolar bone.

placed into osteotomies prepared into the reduced alveolar process when placement into extraction sites was not possible. Five millimetres of the implant was placed within the surgically reduced alveolar ridge to the level of the reference notch, creating 5-mm, critical-size, supra-alveolar, peri-implant defects. Six animals received implants coated with rhBMP-2 at 0.75 or 1.5 mg/ml in contralateral jaw quadrants, and six animals received implants coated with rhBMP-2 at 3.0 mg/ml or uncoated controls using the same split-mouth design. Treatments were randomized between left and right jaw quadrants (Table 1). The periostea of the mucogingival flaps were fenestrated at the base of the flaps to allow tension-free flap apposition and wound closure. The flaps were advanced 3–4 mm coronal to the implants and the flap margins adapted and sutured (GORE-TEX™ Suture CV5, W. L. Gore & Associates Inc., Flagstaff, AZ, USA). Photographic registrations were obtained following implant placement and wound closure.

The maxillary first, second and third premolar teeth were surgically extracted and the maxillary fourth premolars reduced in height and exposed pulpal tissues sealed (Cavit®, ESPE, Seefeld/Oberbayern, Germany) in order to alleviate potential trauma from the maxillary teeth to the experimental mandibular sites.

#### Post-surgery procedures

A long-acting opioid, buprenorphine HCl (0.01–0.03 mg/kg IM) was administered immediately post-surgery and redosed twice daily for 3 days. A broad-spectrum antibiotic (enrofloxacin, 2.5 mg/kg IM) was administered immediately post-surgery and redosed twice daily for 7 days. Sutures were removed under sedation (propofol, 5–7 mg/kg IV) at approximately 10 days. Radiographs were obtained under sedation (propofol, 5–7 mg/kg IV) immediately post-surgery (baseline), and at weeks 4 and 8 post-surgery. Plaque control was maintained by daily flushing of the oral cavity with chlorhexidine gluconate (Xttrium Laboratories Inc., Chicago, IL, USA; 20–30 ml of a 2% solution) until completion of the study. Observations of experimental sites with regards to gingival health, maintenance of suture line closure, oedema and evidence of tissue necrosis or infection were recorded daily.

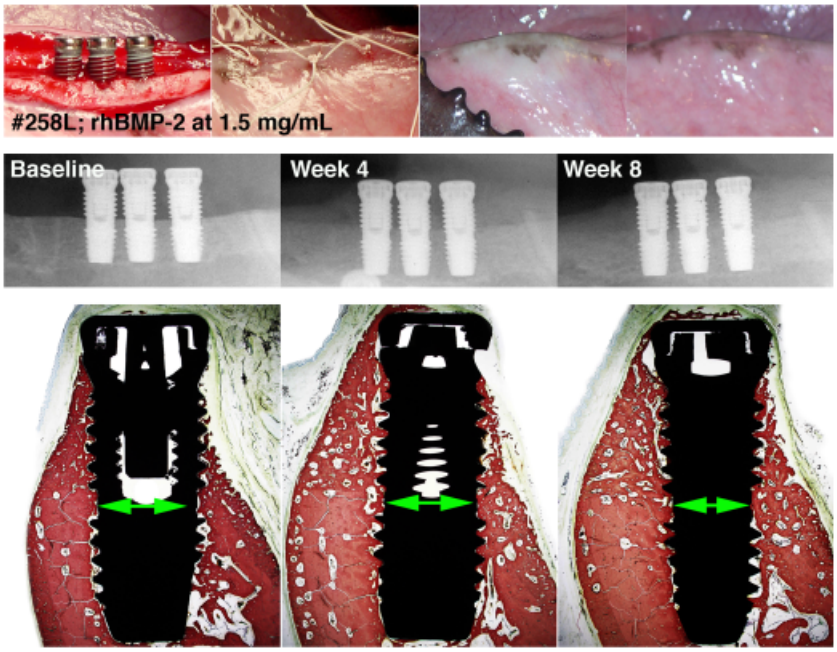


*Fig. 3*. Clinical photographs show ø 4.0 × 10 mm implants coated with recombinant human bone morphogenetic protein-2 (rhBMP-2) at 1.5 mg/ml following placement and wound closure, and healing at weeks 4 and 8. The implant platforms (cover screws) can barely be detected below the mucosa at week 8. Radiographs show limited peri-implant radiolucencies (seromas) at week 4 apparently resolving at week 8. The photomicrographs show bone formation with an established cortex reaching or exceeding the implant platform. Green arrows delineate the 5-mm notch placed level with the resident alveolar bone.

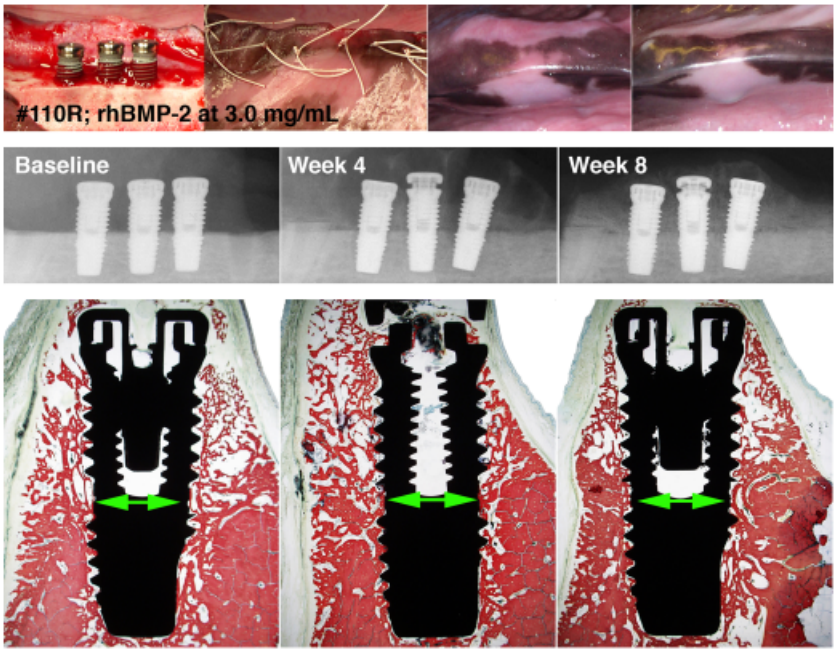


*Fig. 4*. Clinical photographs show ø 4.0 × 10 mm implants coated with recombinant human bone morphogenetic protein-2 (rhBMP-2) at 3.0 mg/ml following placement and wound closure, and healing at weeks 4 and 8. Note significant swelling at week 4 somewhat resolving week 8. Radiographs show significant peri-implant radiolucencies (seromas) at week 4 apparently resolving week 8. Note the partial loosening of a cover screw within the tissues and implant displacements. The photomicrographs show bone formation without an established cortex exceeding the implant platform. Note the partial loosening of a cover screw within the tissues at the central implant. Green arrows delineate the 5-mm notch placed level with the resident alveolar bone.

Fluorescent bone labels were used to evaluate bone formation dynamics (Li & Jee 2005). Oxytetracycline hydrochloride (Maxim-200, Phoenix Pharmaceuticals, St. Joseph, MO, USA; 25 mg/kg SQ) was administered at week 3, xylenol orange (Sigma-Aldrich Inc., St. Louis, MO, USA; 200 mg/ml; 90 mg/kg SQ, twice 1 day apart) at week 4 and calcein (Sigma-Aldrich Inc.; 25 mg/ml; 5 mg/kg, SQ) at days 10 and 3 pre-euthanasia.

The animals were anesthetized and euthanized at week 8 post-surgery by an IV injection of concentrated sodium pentobarbital (Euthasol®, Delmarva Laboratories Inc., Midlothian, VA, USA). Following euthanasia, block sections including implants, alveolar bone and surrounding mucosa were collected and radiographed.

### Histotechnical procedures

The block sections were fixed in 10% buffered formalin for 3–5 days, dehydrated in alcohol, and embedded in methylmethacrylate resin (Technovit 7200 VLC, Kulzer, Wehrheim, Germany). The implants were cut mid-axially in a buccal–lingual plane into 200-$\mu$m thick sections using the cutting–grinding technique (EXAKT Apparatebau, Norderstedt, Germany), and were subsequently ground and polished to a final thickness of approximately 40 $\mu$m for fluorescent light microscopy (Donath & Breuner 1982, Rohrer & Schubert 1992). Upon completion of the fluorescent light examination, the sections were stained with Stevenel's blue and van Gieson's picro fuchsin for histopathologic and histometric analysis using incandescent, polarized and fluorescent light microscopy.

### Clinical analysis

One examiner (G. P.) recapped the clinical observations from laboratory notebook entries and clinical photographs with focus on 4- and 8-week observations including whether implants were visible and/or palpable through the mucosa; the cover screw or the body of the implant was exposed to the oral cavity; and whether signs of seroma formation including a reddish-bluish fluctuating swelling that could not be related to an infectious process were noticeable.

*Table 1.* Distribution of treatments among animals receiving implants coated with recombinant human bone morphogenetic protein-2 (rhBMP-2)

| | rhBMP-2 | |
|---|---|---|
| No. of animals | 6 | 6 |
| Test item | rhBMP-2 0.75 *versus* 1.5 mg/ml | rhBMP-2 3.0 mg/ml *versus* control |
| Implants per jaw quadrant | 3 | 3 |
| Healing interval (weeks) | 8 | 8 |

#### Histopathologic analysis

Two masked, experienced examiners (U. M. E. W. and M. Q.) performed the histopathologic evaluation including observations of bone formation and resorption, cortex formation, seroma formation, fibrovascular tissue and marrow, and inflammatory reactions using computer-enhanced images, fluorescent, incandescent and polarized light microscopy (BX 60, Olympus America Inc., Melville, NY, USA).

#### Histometric analysis

One masked, calibrated examiner (M. Q.) performed the histometric analysis using incandescent and polarized light microscopy (BX 60, Olympus America Inc.), a microscope digital camera system (DP10, Olympus America Inc.) and a PC-based image analysis system (Image-Pro Plus™, Media Cybernetic, Silver Spring, MD, USA). The most central section for each implant was used for the histometric analysis of the buccal and lingual surfaces of each implant including:

- *Defect height*: distance between the reference notch and the implant platform.
- *Bone regeneration (height)*: distance between the reference notch and the vertical extension of newly formed bone along the implant, excluding bone formation exceeding the implant platform.
- *Bone regeneration (area)*: area of newly formed bone along the implant above the reference notch, excluding bone formation exceeding the implant platform.
- *Bone density (new bone)*: ratio bone/marrow spaces in newly formed bone.
- *Osseointegration (new bone)*: percent BIC as measured between the reference notch and the vertical extension of newly formed bone along the implant.
- *Bone density outside the implant threads (resident bone)*: ratio of bone/marrow spaces in a $300 \times 1800\ \mu m$ area (width $\times$ height) immediately outside the implant threads in resident bone.
- *Bone density within the implant threads (resident bone)*: ratio of bone/marrow spaces within the implant threads in resident bone.
- *Osseointegration (resident bone)*: percent BIC within resident bone measured from the reference notch to the apex of the implant.

#### Statistical analysis

The animal was used as the unit of analysis. All the measurements at site level were averaged for each jaw quadrant. A general linear model including a population-averaged panel-data methodology to account for the split-mouth design was used. A robust variance estimation was employed in these models. Analysis of differences between doses was performed using Wald tests adjusted for multiple comparisons. The level of significance was set at 5%. Examiner reliability was assessed using the concordance correlation coefficient (Lin 1989, 2000), which ranges between 0 and 1. Concordance correlation coefficient for linear measurements of bone height was 0.96 showing high reliability. All the analyses were performed using a computer-based statistical software (Stata 7.0 for Windows, Stata Corporation, College Station, TX, USA).

## Results

#### Clinical observations

Early healing events were generally uneventful. All the jaw quadrants receiving implants coated with rhBMP-2 or uncoated control implants healed without wound failure; however, jaw quadrants receiving implants coated with rhBMP-2 exhibited significant swelling compared with the uncoated control (Figs 1–4). Healing through week 8 progressed uneventfully. No implant was lost. There were no dramatic differences relative to the number of visible, palpable and exposed implants coated with rhBMP-2 at 0.75, 1.5 or 3.0 mg/ml. Most implants showed new tissue formation hard to palpation covering the implant body. Even though clinical signs of seroma formation were present for a few implants at week 4, none exhibited clinical evidence of seroma formation at week 8 as evidenced by reddish-bluish fluctuating swellings. For the sham-surgery control, all the implants were visible, palpable, and there was no evidence of new hard tissue or seroma formation. The cover screw of several implants was exposed.

Radiographic evidence of bone formation was observed for all the rhBMP-2-coated implants by week 4. The induced bone increased in density at the 8-week endpoint. Limited, if any, radiographic bone formation was observed in the control group. The radiographic observations in this study are detailed elsewhere (Leknes et al. 2008).

#### Histologic observations

Jaw quadrants receiving control implants showed limited, if any, new bone formation (Figs 1 and 5). No implant exhibited seroma formation. Noteworthy, a large number of implants exhibited remodelling of buccal crestal plate resulting in a net loss of crestal bone.

Jaw quadrants receiving implants coated with rhBMP-2 at 0.75 and 1.5 mg/ml exhibited robust bone formation approaching and/or exceeding the implant platform, bone formation generally being greater at the lingual aspect of the implants (Figs 2, 3 and 5). There were no noteworthy differences in appearance between the newly formed and the immediately adjacent resident bone other than areas of recovering seromas exhibiting a more immature trabecular bone than generally observed. The quality of the newly formed bone approximated that of the adjoining Type II resident bone. Cortex formation was observed already at the 8-week time point. A majority of the implants exhibited peri-implant bone remodelling extending to or beyond $300\ \mu m$ from the implant surface including remodelling of the buccal crestal plate (Fig. 5).

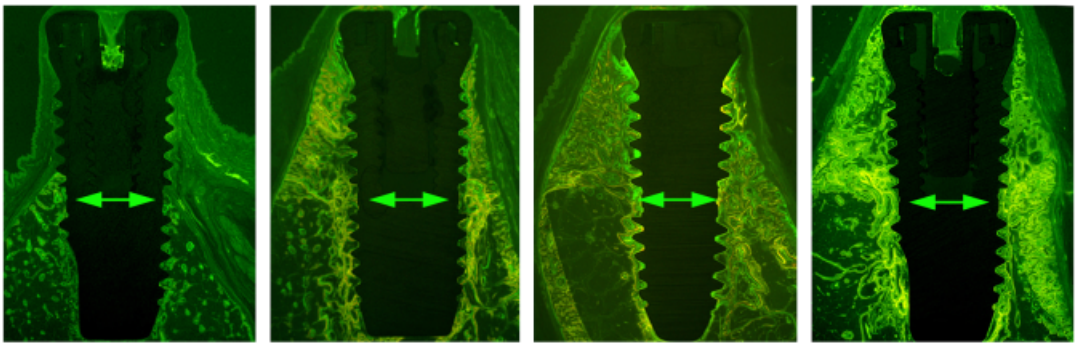

*Fig. 5*. Fluorescence photomicrographs show ø 4.0 × 10 mm implants coated with recombinant human bone morphogenetic protein-2 (rhBMP-2) and sham-surgery control. Control implants exhibit limited new bone formation (left), whereas implants coated with rhBMP-2 at 0.75 (left centre), 1.5 (right centre) and 3.0 mg/ml (right) exhibit bone formation reaching or exceeding the implant platform. Wide yellow and orange fluorescent labels throughout the newly formed bone indicate rapid early bone formation *versus* that observed in the control indicating limited, if any, bone formation. Gradually increasing peri-implant bone remodelling in particular involving the buccal alveolar plate can be observed with increasing rhBMP-2 concentrations. Green arrows delineate the 5-mm notch placed level with the resident alveolar bone.

Dose-related seroma formation was observed limited to one animal receiving implants coated with rhBMP-2 at 0.75 mg/ml *versus* three animals receiving implants coated with rhBMP-2 at 1.5 mg/ml.

Jaw quadrants receiving implants coated with rhBMP-2 at 3.0 mg/ml showed extensive bone formation generally exceeding the implant platform (Figs 4 and 5). Bone appeared immature and without cortex formation compared with that observed for implants coated with lower rhBMP-2 doses. All but one jaw quadrant exhibited expansive seroma formation extending into the resident bone displacing the implants. Advanced seroma formation prevented meaningful histological preparation in two animals. All the implants exhibited extensive peri-implant bone remodelling extending up to or beyond 300 $\mu$m from the implant surface including remodelling of the buccal crestal plate (Fig. 5).

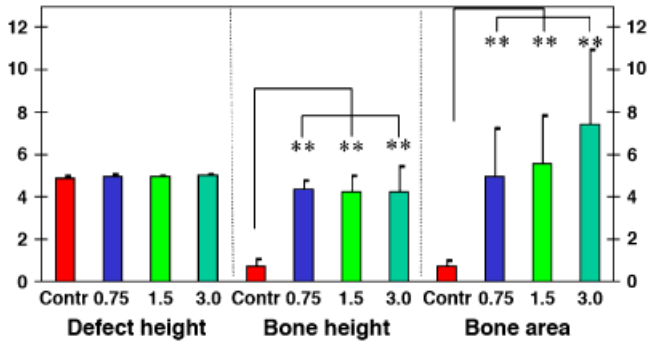


*Fig. 6*. Mean (± SD in mm/mm$^2$) induced bone formation (height and area) for animals receiving uncoated implants (control) or implants coated with recombinant human bone morphogenetic protein-2 (rhBMP-2) at 0.75, 1.5 or 3.0 mg/ml.

### Histometric analysis

Implants coated with rhBMP-2 at 0.75, 1.5 and 3.0 mg/ml exhibited clinically significant bone formation (height and area) compared with the sham-surgery control averaging (± SD) 4.4 ± 0.4, 4.2 ± 0.7 and 4.2 ± 1.2 *versus* 0.8 ± 0.3 mm; and 5.0 ± 2.2, 5.6 ± 2.2 and 7.4 ± 3.5 *versus* 0.7 ± 0.3 mm$^2$, respectively ($p < 0.01$; Fig. 6). Notably, seroma formation increased the induced bone area in animals receiving implants coated with rhBMP-2 at 3.0 mg/ml. Extensive seroma formation and significant remodelling of the resident bone resulted in severe displacement of implants coated with rhBMP-2 at 3.0 mg/ml in two animals disallowing meaningful histometric evaluations.

Bone density in newly formed bone averaged 63%, 61% and 42% for the 0.75, 1.5 and 3.0 mg/ml concentration, respectively (Fig. 7); sites receiving implants coated with rhBMP-2 at 3.0 mg/ml exhibited significantly lower bone density than the other rhBMP-2 groups ($p < 0.05$). Bone density within the limited amount of new bone in the control group (73%) was significantly greater than that of the rhBMP-2-induced bone ($p < 0.01$). Implants coated with rhBMP-2 also exhibited significantly smaller BIC values ranging from 30% to 39% *versus* 79% for the sham-surgery control ($p < 0.01$).

There were significant differences in the resident bone density immediately outside the threads at sham-surgery control implants (77%) compared with the remodelled bone at implants coated with rhBMP-2 at 0.75 mg/ml (66%), 1.5 mg/ml (68%) and 3.0 mg/ml (59%) ($p < 0.05$; Fig. 8). Bone density within the thread area was not different between the control (50%) and that at implants coated with rhBMP-2 at 0.75 (47%) or 1.5 mg/ml (47%), but was significantly lower for implants coated with rhBMP-2 at 3.0 mg/ml (35%; $p < 0.05$). Notably, sham-surgery control implants exhibited significantly higher BICs (78%) compared with implants coated with rhBMP-2 averaging 41%, 41% and 35% for the 0.75, 1.5 and 3.0 mg/ml concentration, respectively ($p < 0.01$).

## Discussion

BMPs have been shown to be safe and to induce clinically relevant bone formation in a variety of settings in the axial and appendicular skeleton (Friedlaender et al. 2001, Wikesjö et al. 2001, Valentin-Opran et al. 2002, Huang et al. 2008), and have subsequently been approved for the clinical use for orthopedic and craniofacial indi-

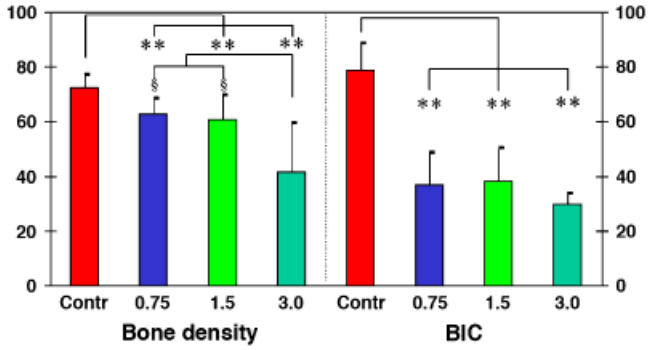


*Fig. 7.* Mean (± SD in %) induced bone density and bone–implant contact (BIC) for animals receiving uncoated implants (control) or implants coated with recombinant human bone morphogenetic protein-2 (rhBMP-2) at 0.75, 1.5 or 3.0 mg/ml.

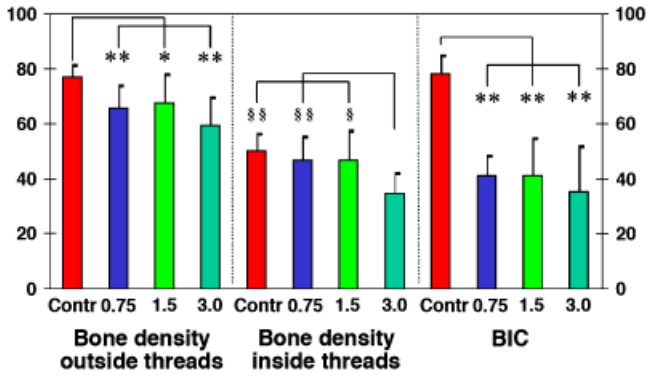


*Fig. 8.* Mean (± SD in %) resident bone density and bone–implant contact (BIC) for animals receiving uncoated implants (control) or implants coated with recombinant human bone morphogenetic protein-2 (rhBMP-2) at 0.75, 1.5 or 3.0 mg/ml.

cations. The rhBMP-2 has been applied to skeletal sites using a variety of carrier technologies (Seeherman & Wozney 2005). For example, rhBMP-2 in an absorbable collagen sponge (ACS) carrier has been used to augment alveolar sites before placement of endosseous oral implants (Hanisch et al. 1997b, Jovanovic et al. 2003) as well as for inlay (Hanisch et al. 2003) and onlay (Sigurdsson et al. 1997, Wikesjö et al. 2003, 2004) indications concomitant with placement of oral implants. rhBMP-2/ACS has been used for subantral augmentation before placement and osseointegration of oral implants (Hanisch et al. 1997b) and has been surgically implanted to establish reosseointegration in advanced peri-implantitis defects (Hanisch et al. 1997a). Endosseous oral implants placed into rhBMP-2-induced bone exhibit significant osseointegration to withstand long-term functional loading (Jovanovic et al. 2003). The present study evaluated an endosseous oral implant coated with rhBMP-2 to show that this novel technology supports clinically relevant local bone formation without the use of adjunctive technologies.

Several model systems have been used to evaluate the biologic and clinical potential of various candidate therapies for craniofacial reconstruction in general and in this context alveolar augmentation and osseointegration. This study used the critical-size, supra-alveolar, peri-implant defect model (Wikesjö et al. 2006). We have shown that critical-size, supra-alveolar, peri-implant defects can reproducibly be created and that with careful wound management, healing will progress without aberrant events such as suture line dehiscencies, exposure and infection of implanted technology. The radiographic and histometric analyses of this model shows consistently limited, if any, regeneration of alveolar bone in sham-surgery control sites over an 8-week healing interval. In other words, the critical-size, supra-alveolar, peri-implant defect model, a genuine onlay defect model, has a limited innate osteogenic potential under optimal conditions for healing. Thus, this discriminating model appears a rigorous and preferred tool in the critical evaluation of candidate technologies including bone biomaterials, devices for guided bone regeneration (GBR) and implantable or injectable technologies using matrix, growth or differentiation factors as stand-alone technologies or in combinations for alveolar augmentation and osseointegration of oral implants.

Implants coated with rhBMP-2 induced bone formation reaching or exceeding the implant platform in this discriminating, 5-mm, critical-size supra-alveolar, peri-implant defect model in a dose-dependent order. The newly formed bone assumed characteristics of the local resident bone for the low (0.75 mg/ml) and mid (1.5 mg/ml) rhBMP-2 concentrations; bone density approximating 60% (Type II bone) and BIC 40%. In other words, the rhBMP-2-coated implants re-established the 5 mm surgically reduced alveolar ridge including cortical plates while providing for clinically relevant osseointegration within an 8-week healing interval. These observations compare favourably with that following the use of GBR technologies with or without the adjunctive demineralized, freeze-dried, allogeneic bone matrix, rhBMP-2 in various concentrations using an ACS carrier with or without space providing devices, or rhBMP-2 in a demineralized, freeze-dried, allogeneic bone matrix. Whereas, GBR treatments marginally support osteogenic bone formation averaging 1–1.5 mm following a 16-week healing interval (Caplanis et al. 1998), rhBMP-2/ACS supports significantly greater, however, highly variable (height/volume), bone formation. Sixteen-week healing intervals combined with higher rhBMP-2 concentrations apparently support denser bone qualities (Sigurdsson et al. 1997), whereas bone formation following shorter healing intervals and lower rhBMP-2 concentrations results in exceedingly irregular (height/volume), sparsely trabecular bone and limited osseointegration of inconsequential relevance (Tatakis et al. 2002). The use of space-providing devices compensates for the irregular bone formation but not for inadequate bone density or osseointegration (Wikesjö et al. 2003, 2004). In contrast, rhBMP-2 in a demineralized, freeze-dried, allogeneic bone matrix rehydrated in autologous blood produces clinically relevant bone formation with respect to height, volume, density and osseointe-

gration (Sigurdsson et al. 2001); however, the public may not continuously embrace the use of cadaver-derived biomaterials as alternatives are/become available. Indeed, synthetic carrier technologies including calcium phosphate matrices have been shown to support clinically relevant alveolar augmentation and osseointegration when combined with rhBMP-2 (Wikesjö et al. 2002) well comparable with that observed for implants coated with rhBMP-2 at 0.75 and 1.5 mg/ml herein.

The high (3.0 mg/ml) rhBMP-2 concentration produced greater bone volumes than the low (0.75 mg/ml) and mid (1.5 mg/ml) concentration, bone formation appearing immature featuring sparsely trabecular woven bone without cortex formation as observed for the lower concentrations. Peri-implant bone remodelling and seroma formation were common for all rhBMP-2-coated implants, however, appeared considerably more extensive or aggressive at the high rhBMP-2 concentration, in two of six animals resulting in grave displacement of the implants most clearly portrayed in the radiographic recordings (Leknes et al. 2008). Similar dose-dependent bone remodelling and seroma formation have been observed in the previous studies evaluating implants coated with rhBMP-2 at 0.2 and 4.0 mg/ml in Type II alveolar bone in the dog (Wikesjö et al. 2008b), or implants coated with rhBMP-2 at 0.2 and 2.0 mg/ml in Type IV alveolar bone in the Cynomolgus monkey (Wikesjö et al. 2008a). Bone remodelling was barely detectable at implants coated with rhBMP-2 at 0.2 mg/ml, however, extending up to 2 mm from the implant surface at implants coated with rhBMP-2 at 2.0 mg/ml in Type IV alveolar bone in the Cynomolgus monkey (Wikesjö et al. 2008a). In contrast, rhBMP-2 at 0.2 mg/ml produced distinct bone remodelling in Type II bone in the dog, bone remodelling invariably greater in extent at sites receiving rhBMP-2 at 4.0 mg/ml (Wikesjö et al. 2008b). Similar to the present study, seroma formation occurred in a dose-dependent order. However, gross implant displacement was not observed probably because the implants were completely inserted into the resident bone and not as in the present study protruding 5 mm above the alveolar crest. Also, the implants were placed more centrally in the alveolar ridge rather than into extraction sites behind a thin, 2-mm buccal cortical plate that readily became subject to remodelling in the absence and presence of rhBMP-2 (Qahash et al. 2008). Importantly, seromas observed in rhBMP-2-induced bone gradually resolve to fill with bone in support of osseointegration and functional loading of oral implants (Sigurdsson et al. 2001, Jovanovic et al. 2003).

There were significant differences in qualities within remodelled resident and rhBMP-2-induced new bone among the low (0.75 mg/ml) and mid (1.5 mg/ml) *versus* the high (3.0 mg/ml) rhBMP-2 concentration. This was not only reflected in bone volume and density but also extended to the presence or absence of cortex formation providing an impression of continuous or delayed bone formation/maturation at implants coated with rhBMP-2 at 3.0 mg/ml. We have in previous studies using the same preclinical model evaluated bone formation following implantation of rhBMP-2/ACS at 0.05, 0.1 and 0.2 mg/ml also following 8-week healing intervals (Tatakis et al. 2002, Wikesjö et al. 2003, 2004). There were no remarkable differences in bone morphology between the various rhBMP-2 concentrations. Bone formation appeared highly irregular and immature, characterized by fine trabecular woven bone and osteoid without cortex formation much like the 3.0 mg/ml rhBMP-2 concentration in the present study. The total dose/site may be estimated to 100, 200 and 400 $\mu$g for the 0.05, 0.1 and 0.2 mg/ml rhBMP-2 concentration, respectively. The corresponding estimations for the rhBMP-2-coated implants are 50, 100 and 200 $\mu$g for the 0.75, 1.5 and 3.0 mg/ml concentration, respectively. Considering that the defect volume for both the study conditions is relatively equal, the observation of dense Type II bone including cortex formation and clinically relevant osseointegration consistently reaching the implant platform at implants coated with rhBMP-2 at 0.75 mg/ml suggests that this technology may provide a relevant release/exposure/retention of rhBMP-2 and that at a considerably low dose. Perhaps, comparably immature bone formation using higher doses of rhBMP-2 simply is a reflection of exposure or release of excessive amounts of rhBMP-2 to the site.

In conclusion, the results from this study suggest that rhBMP-2 coated onto titanium porous oxide implant surfaces induced clinically relevant local bone formation including vertical augmentation of the alveolar ridge and osseointegration. Higher concentrations/doses were associated with untoward effects.

## Acknowledgements

The authors recognize Milton April, DVM, and Alexis Agelan, DVM, for expert veterinary care; Lewis Thomas Bright and Garen Lindsay for excellent animal technical care and animal husbandry; Ms Joanne Drew for terrific administrative support; Hari S. Prasad, BS, MDT, for expert histotechnical preparations; and Knut N. Leknes, DDS, PhD, for thoughtful review of the manuscript. Wyeth Research, Cambridge, MA, USA, kindly donated the rhBMP-2.

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**Clinical Relevance**

*Scientific rationale for the study:* This study is the fourth in a series describing pre-clinical development of a titanium implant surface that combined with rhBMP-2 induces significant local bone formation for optimal implant placement and osseointegration without use of bone grafting, biomaterials or devices for GBR.

*Principal findings and practical implications:* Using the supra-alveolar peri-implant defect model, we show that a titanium porous oxide surface implant coated with rhBMP-2 promotes immediate peri-implant bone formation (vertical augmentation of the alveolar ridge) including clinically relevant osseointegration in a dose-dependent order.